Small Molecules Targeting G4C2 G-Quadruplexes Mitigate RAN Translation in C9orf72 ALS

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Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are progressive, fatal neurodegenerative diseases. A significant portion of ALS/FTD cases are caused by a hexanucleotide repeat expansion (GGGGCC, or G4C2) in the first intron of the *C9orf72* gene. These repeats undergo repeat-associated non-AUG (RAN) translation, producing toxic dipeptide repeat proteins (DPRs) that contribute to neurodegeneration. G4C2 repeats readily form G-quadruplexes which influence RNA-protein interactions, subcellular localization, and may serve as scaffolds or barriers for translation machinery. Here, we investigate the potential of small molecules to bind G4C2 G-quadruplexes, modulate their secondary structure, and mitigate the production of toxic DPRs. To test this, we developed a high-throughput thermal shift assay to screen for small molecules that can modulate the stability of G4C2 G-quadruplexes. We then performed a chemical library screening and identified 48 candidates that stabilized the melting curve of our G4C2 repeat DNA probe. Subsequent testing using a dual luciferase assay showed two compounds emerge as non-toxic and significant inhibitors of RAN translation, reducing DPR levels by up to 75%. These findings were further validated in western blot analysis and mRNA quantification to assess DPR protein abundance and evaluate potential changes in transcript levels. Our results suggest that G-quadruplex stabilization can modulate RAN translation. Ongoing studies aim to uncover the specificity of RNA binding, precise binding pattern, and effects on other disease related mechanisms such as RNA foci formation. Taken together, these data highlight the G4C2 G-quadruplex structure as a therapeutic target which may allow for upstream intervention before the cascade of DPR toxicity.

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